

METARRHIZIUM FLAVOVIRIDE N.SP. ISOLATED FROM INSECTS AND FROM SOIL

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SUMMARY

Metarrhizium flavoviride spec. nov. was isolated from curculionid larvae in Brno and from soil in Kiel-Kitzeberg and Wageningen; it differs from *M. anisopliae* f. *major* by its wider conidia and the greyish yellow-green pigmentation of the conidia. Its pathogenicity to insects has been proved.

All species of *Metarrhizium* so far described are known to be insect parasites, although they have also been isolated occasionally from soil. The fungus known as green muscardine, *M. anisopliae* (Metschn.) Sorokin, dark green with very variable conidial sizes, is polyphagous and cosmopolitan. VEEN (1968) monographed the species, designated a neotype culture (CBS 289.67), compiled a list of 204 host insects (the number of hosts described has increased since), and studied the development of the fungus in insects. DOMSCH & GAMS (1970) compiled the available ecological and physiological information. HAMMILL (1972) investigated the conidium ontogeny with transmission electron microscopy, ZACHARUK (1970a-c, 1971) studied the ultrastructure of conidia, germ tubes and insect penetration structures. Conidia are uninucleate (VEEN 1968; ZACHARUK 1970a; TINLINE 1971); heterokaryosis occurs (TINLINE & NOVIELLO 1971). Information on production of destruxin A, destruxin B and other compounds toxic to insects is accumulating (ROBERTS 1969).

In *M. anisopliae* a distinct forma *major* Johnston can be recognized (JOHNSTON 1915; VEEN 1968) with conidia $7-16 \times 2.5-3.5 \mu\text{m}$ in size (average $11.9 \times 3 \mu\text{m}$), compared with $3-5 \times 2-3 \mu\text{m}$ in f. *anisopliae*. The other two species usually distinguished, *M. album* PETCH (1931) and *M. brunneum* PETCH (1935), are not available as authentic pure cultures and are of uncertain identity; they are possibly identical with *M. anisopliae*. BOROWSKA et al. (1970) described from soil a further species, *M. velutinum*, which, according to the description, is very similar to *M. anisopliae*.

From cultures of larvae and pupae of the curculionids *Ceutorrhynchus macula-alba* Herbst and *C. albovittatus* Germar a strain with yellow-green colonies and relatively wide conidia was isolated in 1956 and recognized as different from *M. anisopliae* (POVOLNÝ & ROZSYPAL 1973). Similar strains were obtained subsequently from agricultural soils in Kiel-Kitzeberg (GAMS et al. 1969) and in Wageningen (J. H. VAN EMDEN, pers. commun.). This fungus is described as a new species.

Metarrhizium flavoviride W. Gams & Rozsypal, *spec. nov.* – Fig. 1a, b. – Coloniae fere lente crescunt, primum albae, deinde griseo-chlorinae, demum dilute olivaceo-bubalinae, pulverulentae vel granulosae. Hyphae vegetativae 2–3 μm crassae. Conidiophora typica sporodochialia laxe verticillata, quorum ramuli phialides binas ad quaternas dense acervatas portant; phialides claviformes, in summo attenuatae, 9–14 μm longae, in parte superiore 3–4.5 μm crassae; conidia longis catenis connexa columnas compactas formant; late ellipsoidea, 7–9(–11) \times 4.5–5.5 μm , levia, fere crassitunicata.

Typus CBS 218.56, isolatus e larvis pupisque *Ceutorrhynchi* maculae-albae, Brno, 1956.

Colonies on malt extract and oatmeal-agar growing rather slowly, at first white, later Greyish Yellow-Green, finally pale Olivaceous Buff (RAYNER 1970); surface mealy and smooth or granular, due to conidial columns (especially on oatmeal-agar); reverse not coloured or ochraceous. Odour indistinct. Octaedric crystals abundantly formed in the medium. Vegetative hyphae smooth, 2–3 μm wide. Conidiophores in fresh strains sporodochial, with loosely verticillate ramification at a rather wide angle, the branches bearing 2–4 phialides which ultimately form a palisade-like layer; in old strains more slender conidiogenous structures occurring solitarily. Phialides in fresh strains (*fig. 1a*) club-shaped, 9–14 μm long, 3–4.5 μm wide at the broadest point, with a strongly tapering and thick-walled conidiogenous apex, in older strains (*fig. 1b*) more slender and cylindrical, 2–3 μm wide. Conidia produced in long chains which form compact columns on the sporodochia; in fresh strains broadly ellipsoidal with faintly differentiated basal end, 7–9 (–11) \times 4.5–5.5 μm , in older strains cylindrical with a rounded upper and a tapering and truncate basal end, 7–10 \times 3–4 μm ; rather thick-walled, smooth, almost hyaline when viewed singly, in mass dilute yellow-green; in fresh strains small vacuoles distributed evenly throughout the conidia, in older ones concentrated near the ends. Chlamydospores absent.

Strains examined:

CBS 218.56 (= IMI 170146) isolated from larvae and pupae of *Ceutorrhynchus macula-alba*, Brno, 1956.

CBS 125.65, 380.73 A and B, isolated from washed mineral particles from deep layers (25–50 cm) of an agricultural soil, grown with wheat after a previous crop of rape, Kiel-Kitzeberg, 1963 and 1964.

CBS 473.73, isolated from agricultural soil, Wageningen, isolated by J. H. van Emden.

Strain 218.56 had lost its pigmentation almost completely after 16 years maintenance at the CBS on oatmeal-agar alternating with rice, but this could partly be restored after several passages over potato blocks. Both CBS 218.56 and CBS 125.65 now show rather narrow cylindrical conidia and few typical sporodochia. The other two strains maintained for almost 10 years in a refrigerator have kept their original shape and abundant sporulation.

M. flavoviride differs from the large-spored forma *major* of *M. anisopliae* (*fig. 1c*) which has narrower, rod-shaped conidia and the same Dark Herbage Green to Dull Green (RAYNER 1970) pigmentation as the forma *anisopliae*.

The aggregation of the conidiophores with often palisade-like crowded phialides should be regarded as sporodochial rather than coremial. Coremia

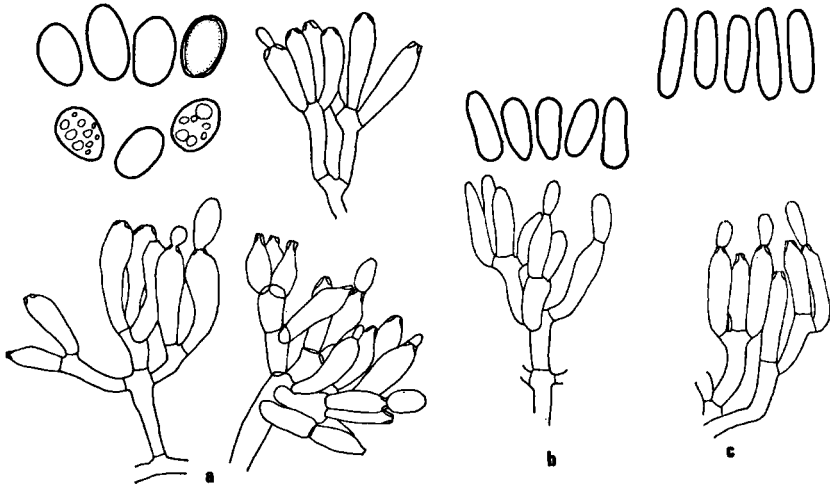


Fig. 1. a, b. *Metarrhizium flavoviride*: a. conidiophores and conidia from fresh strain, b. from old strain. — c. *Metarrhizium anisopliae* f. *major*, conidiophores and conidia. $\times 1000$.

typically produced in some species of *Penicillium* (*Asymmetrica fasciculata* and *Symmetrica*) consist of a fasciculate stalk of conidiophores. In *Metarrhizium* no stalks occur, the erect structures consist only of conidial columns.

Insect pathogenicity of *M. flavoviride* has been proved for strain 218.56 by inoculation on larvae of the two curculionids mentioned and on caterpillars of *Plodia interpunctella* Hübner. CBS 125.65 was inoculated successfully on larvae of *Hyponomeuta malinellus* Zeller and *Malacosoma neustria* L.

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